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Meiotic behaviour of chromosomes in three predator species of the subfamily Asopinae (Heteroptera: Pentatomidae)

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Abstract. The study of chromosome behaviour has been carried out on males of three species of the Asopinae (Pentatomidae) bugs *Andrallus spinidens* (Fabricius, 1787), *Canthecona furcellata* Wolff, 1801 and *Perillus bioculatus* (Fabricius, 1775) collected in India. All the species have XY sex chromosome system and 12 autosomes (2n=14=12A+XY). The general course of meiosis is fairly uniform and is typical for heteropteran species. However, the species differ in the extent of decondensation of the autosomes during the diffuse stage and the number of ring bivalents at diplotene/diakinesis. Also, a metaphase I arrangement of chromosomes which is different from the typical Pentatomidae type has been observed in *C. furcellata* and *P. bioculatus*.

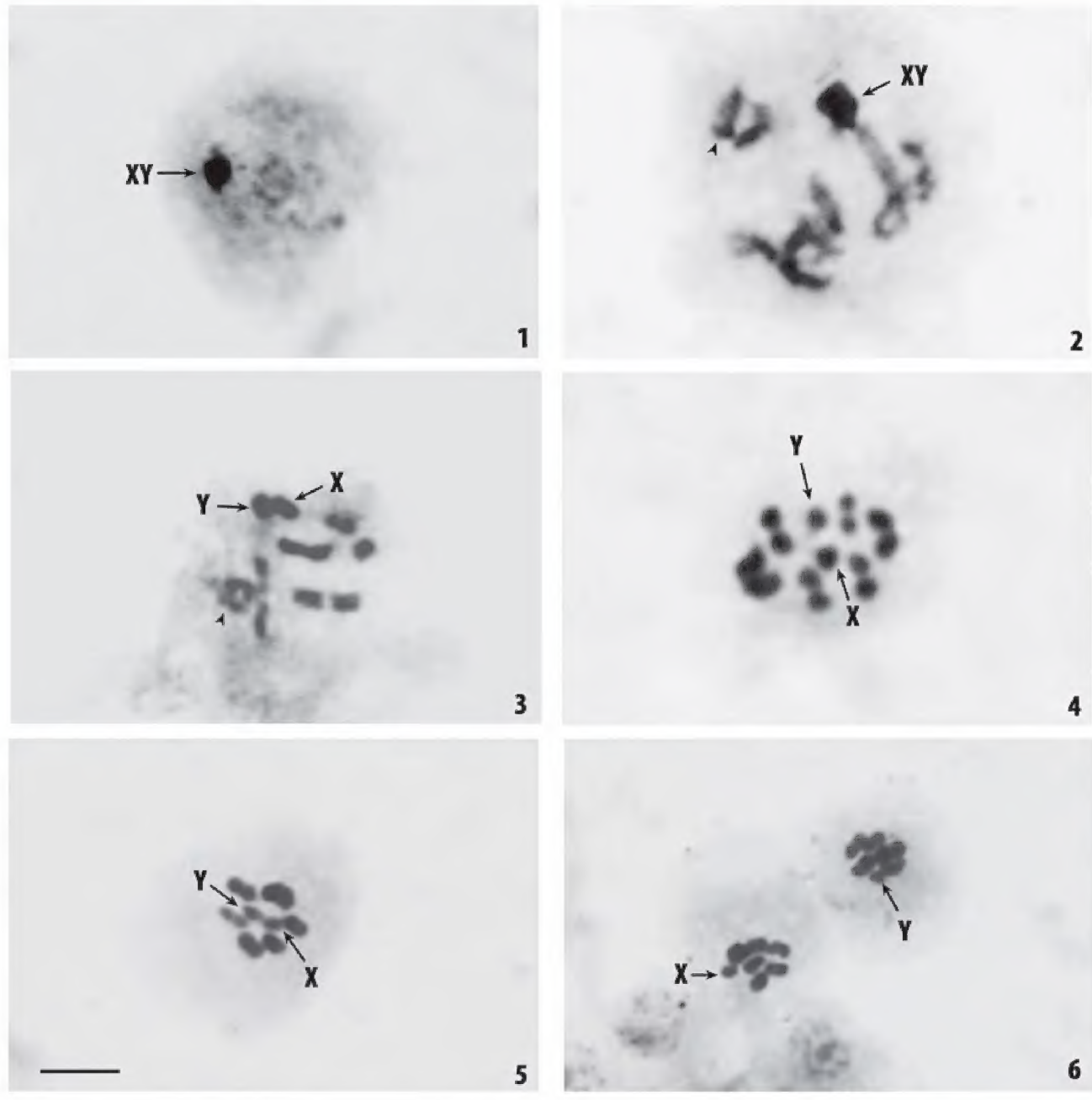
Key words: chromosomes, diffuse stage, metaphase arrangement, Asopinae, Pentatomidae.

Introduction

From cytogenetic point of view, members of the suborder Heteroptera are characterized by possessing holokinetic chromosomes and post-reductional meiosis for sex chromosomes in which the first meiotic division is equational and the second one is reductional (Ueshima, 1979; Kerzhner et al., 2004; Rebagliati et al., 2005). In addition, the meiotic behaviour of autosomal bivalents, sex chromosomes and m-chromosomes is different (Ueshima, 1979; Papeschi et al., 2003).

Pentatomidae are one of the largest families of the Heteroptera with 4123 species (Panizzi et al., 2000) grouped into eight subfamilies (Schuh, Slater, 1995). The Asopinae are a cosmopolitan subfamily of predatory bugs of the family Pentatomidae, comprising 63 genera and 357 species (Schuh, Slater, 1995) and some of them are important agents of biological control (Panizzi et al., 2000). However, only 22 species have been cytologically investigated with a diploid number of 12, 14, 16 or 18 and XY sex determining mechanism. About 80% of the species studied so far possess 2n=14 (Kerzhner et al., 2004; Rebagliati et al., 2005).

This is the first report on the behaviour of meiotic chromosomes in three Indian species belonging to Asopinae (Pentatomidae) *Andrallus spinidens* (Fabricius, 1787), *Canthecona furcellata* Wolff, 1801 and *Perillus bioculatus* (Fabricius, 1775).



Figs 1-6. Male meiosis in *Andrallus spinidens.* **1 -** diffuse stage showing associated and condensed X and Y chromosomes. **2 -** diplotene showing associated sex chromosomes and a single ring bivalent. **3 -** diakinesis with sex chromosomes arranged side by side. **4 -** metaphase I in polar view, sex chromosomes lie inside a ring formed by autosomal bivalents. **5 -** metaphase II, a ring of autosomal univalents with the sex chromosome pseudobivalent in the centre. **6 -** telophase II showing reductionally divided sex chromosomes. Arrowheads indicate ring bivalents; arrows indicate sex chromosomes. Scale bar = 10 μm.

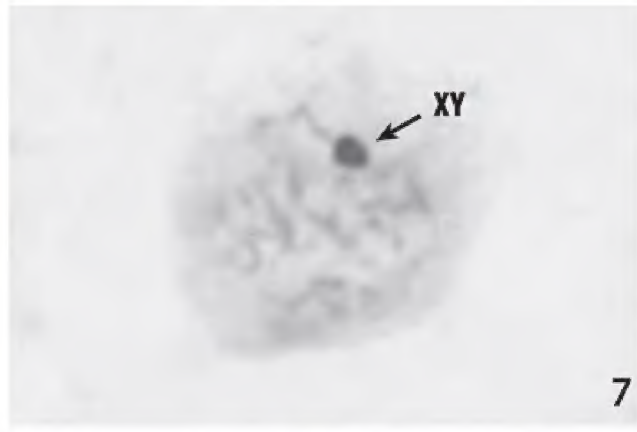
Material and methods

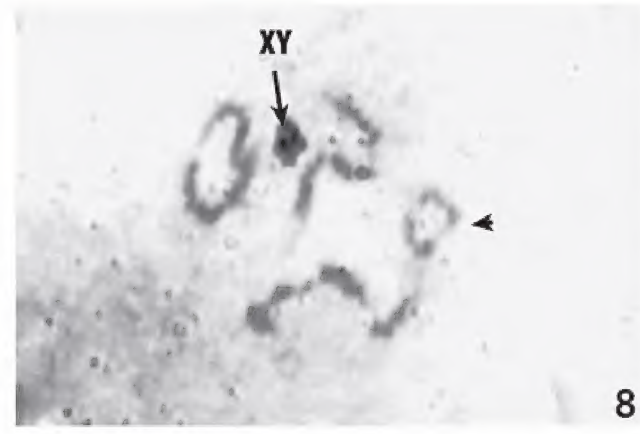
Adult males of *Andrallus spinidens, Canthecona furcellata* and *Perillus bioculatus* were collected from Pathankot (Punjab, India) located at $32^{0}27'$N $75^{0}65'$E coordinates. Testes were removed and fixed in 3:1 ethanol:glacial acetic acid, and air dried chromosomal preparations were stained in basic fuchsin after the protocol suggested by Carr and Walker (1961).

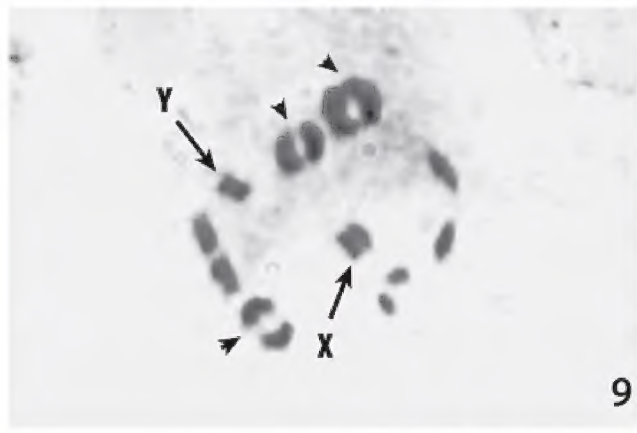
Results

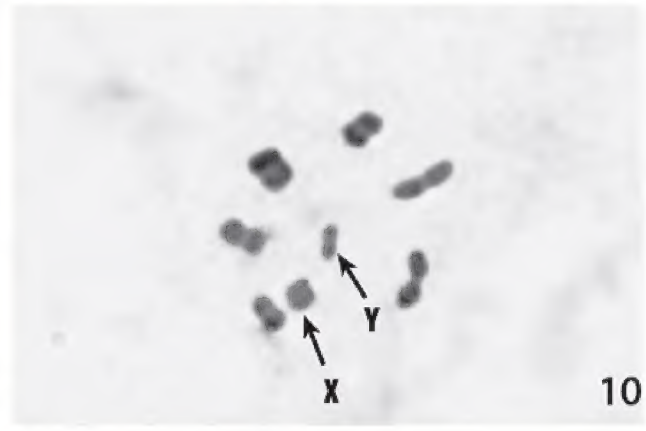
Andrallus spinidens

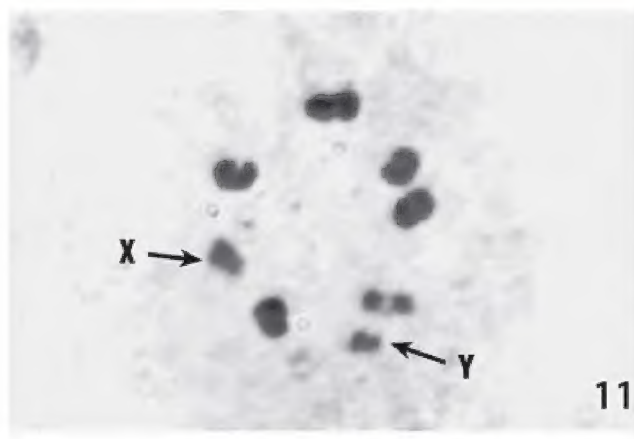
During diffuse stage, the sex chromosomes are associated and form a darkly stained heteropycnotic body while the autosomes are highly decondensed (Fig.1). During diplotene, six autosomal bivalents and closely associated X and Y are seen. One of the autosomal bivalents exists as a ring bivalent (Fig. 2). During diakinesis, the sex chromosomes

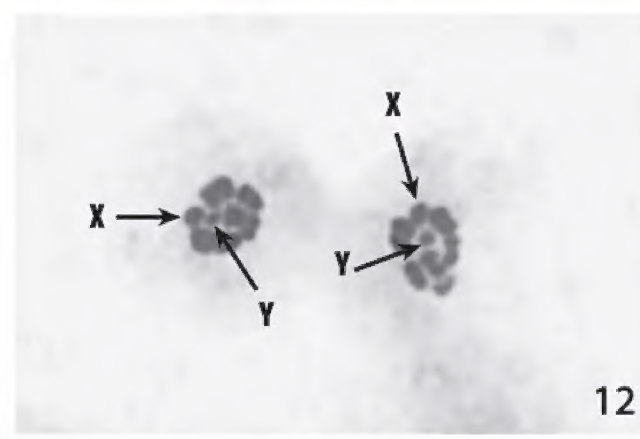


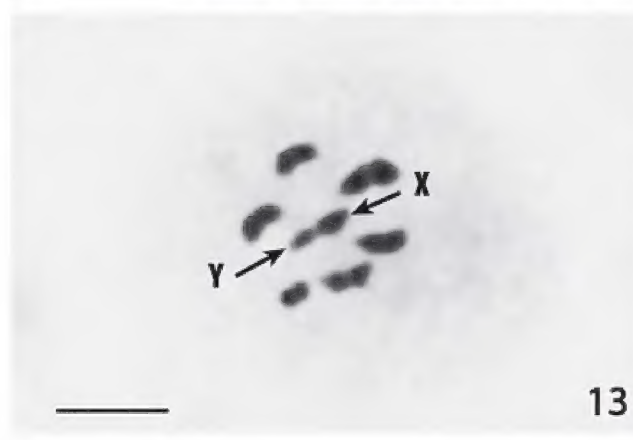






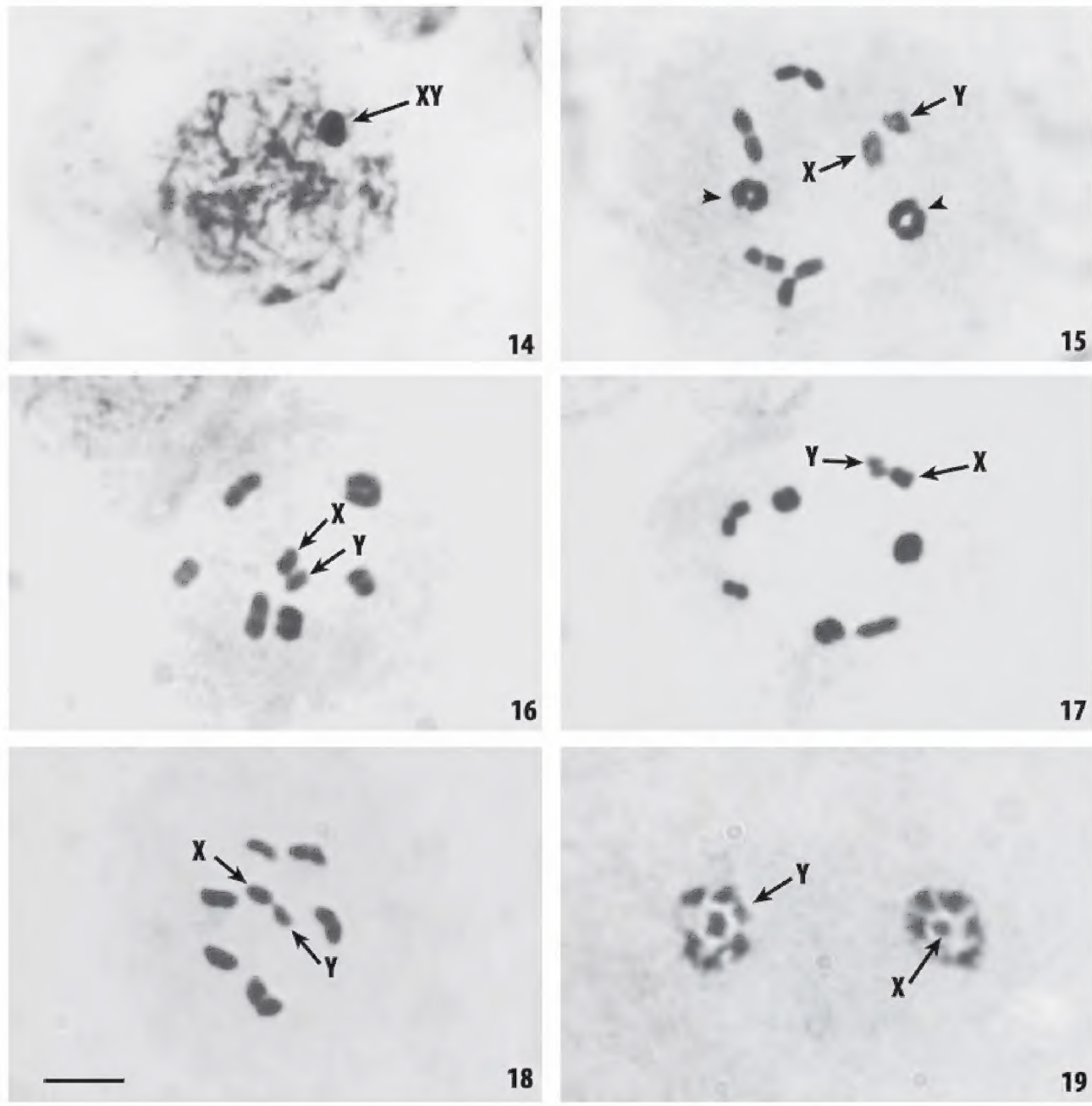






Figs 7-13. Male meiosis in *Canthecona furcellata*. **7** - diffuse stage showing associated X and Y chromosomes and high degree of autosomal decondensation. **8** - diplotene showing two ring bivalents. **9** - diakinesis with three ring bivalents. **10**, **11** - metaphase I stages showing two different arrangements of the chromosomes. **12** - telophase I showing equationally divided sex chromosomes. **13** - metaphase II, a ring of autosomes with strongly associated sex chromosomes in the center. Arrowheads show ring bivalents and arrows show sex chromosomes. Scale bar = 10 μm.

become separated but lie side by side and one of the autosomal bivalents is seen as a ring (Fig. 3). At metaphase I, autosomal bivalents form a circle in the centre of which lie X and Y (Fig. 4). At metaphase II, autosomal univalents form a circle, and X and Y join to form a pseudobivalent that lies in the centre (Fig. 5). At telophase II (Fig. 6), the sex chromosomes



Figs 14-19. Male meiosis in *Perillus bioculatus*. **14** - diffuse stage showing the condensed and paired X and Y chromosomes and partially decondensed autosomes. **15 -** diakinesis showing two ring bivalents and well separated X and Y chromosomes. **16, 17 -** metaphase I plates showing two different arrangements of the chromosomes. **18 -** metaphase II showing a ring of autosomal univalents with weakly associated sex chromosomes. **19 -** telophase II showing reductionally divided sex chromosomes. Arrowheads indicate ring bivalents; arrows indicate sex chromosomes. Scale bar = 10 μm.

divide reductionally, the X and Y move to opposite poles and two types of nuclei, one with the X chromosome and the other with the Y chromosome, are formed.

Canthecona furcellata

At diffuse stage, X and Y are associated and form a darkly stained heteropycnotic body located on one side of the nucleus while the autosomes are completely decondensed (Fig. 7). At diplotene, six autosomal bivalents become distinct due to condensation while sex chromosomes are heteropycnotic and remain together. Two or three autosomal pairs have two terminal chiasmata and form ring bivalents (Fig. 8). These bivalents can be seen also at diakinesis when the sex chromosomes appear as parallel chromatids and are no longer heteropycnotic (Fig. 9). Metaphase I (polar view) shows two types of arrangements. In one, six autosomal bivalents form a ring inside which lie the sex chromosomes. Y is exactly in the centre while X is close to the inner border of the ring (Fig. 10). In the second, all the eight chromosomes arrange in a ring with

an empty centre (Fig. 11). The former is more frequent (10 out of 13 metaphase I counted) than the latter (3 out of 13 metaphase I counts). At telophase I, two nuclei are formed with 8 chromosomes each (six autosomes, X and Y) (Fig. 12). At metaphase II, X and Y form a pseudobivalent that lies in the centre of a ring of autosomes (Fig.13).

Perillus bioculatus

At diffuse stage, X and Y form a darkly stained heteropycnotic body while autosomes are partially decondensed (Fig. 14). Diakinesis is marked by the presence of two ring bivalents which are more densely stained than rest of the bivalents. The sex chromosomes become separated, with more or less parallel chromatids (Fig. 15). Two types of metaphase I plates have been observed. In one, the typical pattern of the family is observed wherein a ring of six autosomal bivalents is formed in the centre of which lie X and Y (Fig. 16). In the second, all the chromosomes (autosomal bivalents and sex chromosomes) form a ring with a hollow centre (Fig. 17). The former pattern is more frequent (16 out of 23 metaphase I counts) than the latter (7 out of 23 metaphase I counts). In both of the patterns, the sex chromosomes lie close to each other. At metaphase II, six autosomal univalents form a ring and sex chromosomes join to form a pseudobivalent which lies in the centre of the ring (Fig. 18). During second meiotic division, sex chromosomes divide reductionally and X goes to one pole and Y to the other (Fig. 19).

Discussion

The general course of meiosis in the three species studied is fairly uniform and behaviour of chromosomes is typical of the Heteroptera. The diploid number of *Perillus bioculatus* is in agreement with the prescription given by Wilson (1906) as 2n=14=12A+XY, and *Andrallus spinidens* and *Canthecona furcellata* also showed the same number which is the modal number for the family Pentatomidae, supporting high karyotypic conservation during the evolution of this group as suggested by Ueshima (1979).

The diffuse stage has been a common meiotic feature in heteropteran species where autosomes are decondensed, sex chromosomes are condensed and the cell size increases (Ueshima, 1979). In Pentatomidae, sex chromosomes usually associate during the diffuse stage although there is much variation with respect to degree of association between X and Y among different species (Rebagliati et al., 2001; Lanzone, Souza, 2006). In the present three predator species, X and Y chromosomes associate closely to form a single heteropycnotic body during the diffuse stage as has been observed in other species of Asopinae (Montgomery, 1906; Heizer, 1950, 1951) and Pentatomidae in general (Camacho et al., 1985; Satapathy, Patnaik, 1988, 1991). The sex chromosomes become separated during diplotene in *Perillus bioculatus* and *Canthecona furcellata.* In *Andrallus spinidens*, however, XY association has been seen extended even up to diplotene stage as is recorded in another predator Pentatomidae species *Oechalia grisea* (Burmeister, 1834) by Heizer (1951).

The degree of decondensation of the autosomes during the diffuse stage varies among different species of Heteroptera – from species with high degree of decondensation to species whose chromosomes do not decondense at all and diffuse stage is absent (Lanzone, Souza, 2006). *Andrallus spinidens* and *C. furcellata* showed high degree of decondensation as was observed in species from different subfamilies of Pentatomidae such as *Nezara viridula* (Linnaeus 1758) of the subfamily Pentatominae (Camacho et

al., 1985) and *Antiteuchus mixtus* (Fabricius, 1787), *A. sepulcralis* (Fabricius, 1803) and *A. macraspis* (Perty, 1834) of the subfamily Discocephalinae (Lanzone, Souza, 2006). In *P. bioculatus,* however, the autosomes showed a partial decondensation, and it is the first record in the family Pentatomidae though this pattern was earlier reported in the families Coreidae (Papeschi, Mola, 1990) and Aradidae (Jacobs, Liebenberg, 2001). The reason for variation in the degree of decondensation and its significance has not been yet established. Differences in the duration of the diffuse stage and level of autosome decondensation are suggested to "correspond to variations in the interval of disintegration between the meiotic and mitotic chromosome structure" (Lanzone, Souza, 2006).

The general trend in Pentatomidae is the predominance of one chiasma per bivalent (Satapathy, Patnaik 1988; Lanzone, Souza 2006). However, in *C. furcellata* and *P. bioculatus*, two to three ring bivalents have been observed while in *A. spinidens*, a single ring bivalent has been seen during prophase I. The presence of more than one chiasma per bivalent is less common in Pentatomidae, and has been reported so far in *Nezara viridula* (Linnaeus, 1758) (Pentatominae) by Camacho et al. (1985) and Papeschi et al. (2003), in *Macropygium reticulare* (Fabricius, 1803) (Discocephalinae) and *E. meditabunda* (Fabricius, 1794) (Edessinae) by Rebagliati et al. (2001, 2003). Our results have added three more species to this category.

In most of the Pentatomidae species, metaphase I is characterized by a ring of autosomal bivalents in the centre of which lie the X and Y univalents (Manna, 1951; Satapathy, Patnaik, 1988; Rebagliati et al., 2001; Lanzone, Souza, 2006). This behaviour has been observed in the three species analysed here. However, in *C. furcellata* and *P. bioculatus*, another arrangement of chromosomes has been observed less frequently (in 23.1% and 26.1% of metaphase I counted, respectively), in which all the chromosomes form a ring with an empty centre. Deviations from the typical arrangement have also been reported in two species of subfamily Pentatominae viz., *Carbula socia* (Walker, 1867) (Satapathy, Patnaik, 1988) and *Eurydema pulchrum* (Westwood, 1837) (Kaur et al., 2006) where all the chromosomes are randomly arranged on the metaphase I plates.

In Pentatomidae, metaphase II is radial where autosomal univalents form a ring in the centre of which lies the end-to-end associated XY pseudobivalent (Manna, 1951; Parshad, 1957; Satapathy, Patnaik, 1988; Rebagliati et al., 2001). Same results have been observed in all the three species analysed in this work.

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